PATENT
Docket No. 56446-2006600
Client Docket No. D1440-2US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Mark MADDEN, et al.

Serial No.: 09/751,299

Filing Date: December 28, 2000

For: METHODS FOR PRODUCING ALPHA-SUBSTITUTED CARBOXYLIC ACIDS USING NITRILASES AND STRECKER REAGENTS (AS AMENDED)

Examiner: K. Kerr

Group Art Unit: 1652

DECLARATION OF GRACE DESANTIS, PH.D. PURSUANT TO 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Grace DeSantis, declare as follows:

1. I am an expert in the field of molecular biology and enzyme development and was an expert at the time of the invention. I am presently employed as a research scientist at Diversa Corporation, San Diego, California, assignee of the above-referenced application. My resume is attached as documentation of my scientific credentials.

2. I declare that the state of the art at the time of the invention and the level of skill of a person of ordinary skill in the art, *e.g.*, determining substrate reactivity, for nitrilase

EXHIBIT A

enzymes, was very high. It would not have required any knowledge or guidance beyond that provided in the specification as to which substrates are useful with the specific claimed enzymes. At the time of the invention, methods for screening for enzyme substrates were sufficiently comprehensive, routine and predictable at the time of the invention to easily identify which the aldehydes, ketones, cyanide-containing compounds, and ammonium containing compounds to stereoselectively produce an α-substituted carboxylic acid using nitrilases encoded by SEQ ID NO:2 and SEQ ID NO:4.

3. I declare that the specification provides sufficient guidance for the person of ordinary skill to identify the claimed subgenus of nitrilases. Methods for sequence modifications were sufficiently comprehensive, routine and predictable at the time of the invention to predictably generate nitrilase-encoding sequences or protein sequences without need of knowing which specific regions of nitrilase sequence or structure affected nitrilase function or activity. Methods known at the time of the invention for modifying nucleic acid or protein sequences in combination with high through-put enzyme activity screening known at the time of the invention, made methods that require previous knowledge of protein structure, including secondary or tertiary structure, active site sequences, and the like obsolete and unnecessary. Using methods known in the art at the time of the invention it would not have been necessary to understand which specific regions of nitrilase structure needed to be modified to generate a genus of nucleic acids or polypeptides for practicing the invention without undue experimentation. The specification provides an exemplary assay in Example 1. Using the disclosed methods, it is routine to screen sequences with 70% identity to SEQ ID NO:2 or SEQ ID NO:4 for reactivity with the disclosed class of substrates.

4. I am a co-author of the Robertson et al., *Applied Environ. Microbiol.* 70:2429-36 (2004) article and participated in the disclosed experiments and analysis. I declare that Robertson demonstrates the *routine* nature of determining substrate specificity. *One hundred and thirty-seven* nitrilases disclosed were identified using a set of only two nitrilase substrates, *i.e.*, adiponitrile and (*R,S*)-4-chloro-3-hydroxyglutaronitrile to screen the clones in a high throughput format. Such experiments demonstrate the routine nature of screening for substrate reactive to a particular substrate, the predictability of such screening, and ease of determining

positive results. Therefore, no undue experimentation would be required given the specification's disclosure of a specific nucleic acid structure for the claimed enzymes, a specific and routinely used physio-chemical property of claimed subgenus, *i.e.*, 70 % sequence identity, a defined function, *i.e.*, nitrilase activity, using a well known assay and a defined class of substrates. Accordingly, the specification provides sufficient guidance to one of ordinary skill in the art to make and use the genus of nitrilases.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Executed at San Diego, California, on April 8, 2005.

Grace DeSantis, Ph.D.

Grace DeSantis, Ph. D.

Work:	*Home:*
Diversa Corporation	
4955 Directors Place	**8290 Torrey Gardens Place**
San Diego, CA	**San Diego, CA**
92121	**92129**
Phone: (858) 526-5471	**Phone: (858) 484-3319**
e-mail: gdesantis@diversa.com	**grace_desantis@hotmail.com**

Education:

May 2002 — Certificate Program, Medicinal Chemistry. University of California, San Diego.

Jan. 1999 — **Ph. D**. University of Toronto, Bio-Organic Chemistry.
Thesis Title: "Tailoring the Specificity of subtilisin *Bacillus lentus* via a Combined Site-Directed Mutagenesis and Chemical Modification Approach."
Advisor: Professor J. B. Jones.

Jan. 1996 — **M. Sc**. University of Toronto, Bio-Organic Chemistry.
Thesis Title: "Inhibition of α–Chymotrypsin and subtilisin Carlsberg by *N*-Oxamide, α-Keto amide and *N*-Succinimidyl Derivatives." Advisor: Professor J. B. Jones.

Jun. 1994 — **B. Sc**. University of Toronto, Honors with Distinction. Biochemistry and Chemistry.

Experience:

02/2003 – present — Senior Staff Scientist, Integrated Chemical Process: Diversa Corporation.

04/2001 –02/2003 — Staff Scientist II, Integrated Chemical Process: Diversa Corporation.

- Project leadership and management.
- Manage group focused on assay development and enzymology.
- Protein engineering.
- High throughput screening.
- Discovery and evolution of novel enzymes.
- Led a project team that focused on the integration of biocatalysis and chemical catalysis for the development of route to pharmaceutical intermediate which was licensed for commercialization development.
- Contributed to patent applications and invention disclosures.
- Published two first author papers describing the utility of the discovered nitrilase platform.

04/1999 – 04/2001 — Postdoctoral Fellow (NSERC) The Scripps Research Institute, Department of Chemistry.
Advisor: Professor C.-H. Wong

- Design of an *in vivo* selection assay to evolve aldolase specificity
- Enzymes in organic synthesis
- Enzyme engineering and screening
- Protein expression and purification, Molecular biology.

1996-1999 — Ph. D. Research Student (NSERC), University of Toronto, Department of Chemistry.
Advisor: Professor J. B. Jones.

- Design and Synthesis of enzyme substrates and inhibitors
- Kinetic evaluation of substrate and inhibitor binding

- Subjected engineered proteases to site specific chemical modification
- Enzyme assays
- Protein purification and characterization: PAGE, ES-MS, FPLC, HPLC.

1997-1998 Tutor for Introductory Organic Chemistry Course: Supervisor: Dr. S. Skonieczny

1996-1997 Marker for Biological Chemistry Course: Supervisor: Professor A. M. MacMillian
1994-1995 Supervisor: Professor J. B. Jones

1994-1996 M. Sc. University of Toronto, Department of Chemistry.
Advisor: Professor J. B. Jones

- Design and synthesis of transition state analogue inhibitors of serine proteases
- Kinetic evaluation of inhibitor potency with serine proteases.

1994-1996 Laboratory Demonstrator Second Year Advanced Organic Chemistry Course.
University of Toronto, Department of Chemistry. Supervisor Dr. S. Skonieczny

1993-1994 B. Sc. Research Student, University of Toronto, Department of Chemistry.
Advisor: Professor. J. B. Jones. Fourth year thesis Project

- Synthesis of transition state analogue inhibitors
- Kinetic evaluation of chiral aldehyde inhibitors of serine proteases

05/1993 –08/1993 Summer Research Student (NSERC fellow), University of Toronto, Department of
05/1992 –08/1992 Biomaterials, Advisor Professor J. M. Lee.

- Designed a research project to correlate structure and function relationship of pericardial tissue by immuno-localization of collagen type I and III
- Harvesting of cardiac tissue from various animal subjects
- Chemical treatment to stabilize and modify protein crosslinks
- Biochemical analysis

Special Skills and Interests:

- Proficiency with Microsoft Word, Microsoft Excel, ChemDraw. Experience with Accelrys Suite of Molecular modeling software.
- Experience with: HPLC, GC, NMR, PAGE, pH-Stat, UV/VIS kinetics.
- Enzymology, Biocatalysis, Assay development, ELISA, Organic Chemistry, Medicinal chemistry.
- Additional Languages: French and Italian

Honors and Awards:

2001	Bio-Mega/Boehringer Ingelheim Award for Organic or Bioorganic Chemisry, 84th CSC
2000	Co-recipient of Presidential Green Chemistry Challenge Award honoring C.H. Wong
1999-2001	Natural Science & Engineering Research Council of Canada, Postdoctoral Fellowship
1996-98	Natural Science & Engineering Research Council of Canada, PGS II Fellowship
1995-96, 1998	University of Toronto Open Fellowship
1994	Dr. James A. & Connie P. Dickson Scholarship
1994	Graduate with Distinction, University of Toronto
1993	Natural Science and Engineering Research Council of Canada, Summer Fellowship
1990	Ontario Government Scholar and Outstanding Student Award
1989	Canada Day Award for Excellence (Federal Government)

Scientific Publications:

1.) M. R. Stabile, W.G. Lai, G. DeSantis, M. Gold, and J. B. Jones, C. Mitchinson, R. R. Bott, T. P. Graycar, and C.-C. Liu **Probing the Specificity of the S_1 Binding Site of M222 Mutants of Subtilisin *B. lentus* with Boronic Acid Inhibitors**. *Bioorg. Med. Chem. Lett*, **1996**, 6(21), 2501-2506.

2.) P. Berglund, G. DeSantis, M.R. Stabile, X. Shang, M. Gold, R.R. Bott, T.P. Graycar, T.H. Lau, C. Mitchinson, and J.B. Jones **Chemical Modification of Cysteine Mutants of Subtilisin *B. lentus* Can Create Better Catalysts than the Wild-type Enzyme**. *J. Am. Chem. Soc.* **1997**, 119(22) 5265-5266.

3.) G. DeSantis, P. Berglund; M. R. Stabile, M. Gold, and J. B. Jones **Site-Directed Mutagenesis Combined with Chemical Modification as a Strategy for Altering the Specificity of the S_1 and S_1' Pockets of Subtilisin *Bacillus lentus*.** *Biochemistry* **1998**, 37, 5968-5973.

4.) G. DeSantis, and J. B. Jones **Chemical Modification at a Single Site Can Induce a Significant Shift in the pH Profile a Serine Protease** *J. Am. Chem. Soc.*, **1998**, 120, 8582-8586.

5.) J. B. Jones, G. DeSantis **Towards Understanding and Tailoring the Specificity of Synthetically Useful Enzymes**. [Invited Review] *Acc. Chem. Res.* **1999**, *32*, 99-107.

6.) G. DeSantis, J. B. Jones **Probing the Altered Specificity and Catalytic Properties of Mutant Subtilisin Chemically Modified at Position S156C and S166C in the S_1 Pocket.** *Bioorg. Med. Chem.* **1999**, 7, 1381-1387.

7) E. Plettner, G. DeSantis, M. R. Stabile, J.B. Jones **Modulation of Esterase and Amidase Activity of Subtilisin *Bacillus lentus* by Chemical Modification of Cysteine Mutants.** *J. Am. Chem. Soc.* **1999**, *121*, 4977-4981.

8.) G. DeSantis, J. B. Jones **Chemical Modification of Enzymes for Enhanced Functionality**. [Invited Review] *Current Opin. Biotech.* **1999**, *10(4)*, 324-330.

9.) K. Khumtaveeporn, G. DeSantis, J. B. Jones **Expanded Structural and Stereospecificity in Peptide Synthesis with Chemically Modified Mutants of Subtilisin**. *Tetrahedron: Asymmetry,* **1999**, *10(13)*, 2563-2572.

10.) G. DeSantis, X. Shang, J. B. Jones **Towards Tailoring The Specificity of the S_1 Pocket of Subtilisin *B. lentus*: Chemical Modification of Mutant Enzymes as a Strategy for Removing Specificity Limitations.** *Biochemistry,* **1999**, *38(40)*, 13391-13397.

11.) B. G. Davis, X. Shang, G. DeSantis, R. R. Bott, J. B. Jones **The Controlled Introduction of Multiple Charges at a Single Site in Subtilisin *Bacillus lentus*.** *Bioorg. Med. Chem* (1999), 7(11), 2293-2301.

12.) G. DeSantis, C. Paech, J. B. Jones **Benzophenone Boronic Acid Photoaffinity Labeling of Subtilisin**. *Bioorg. Med. Chem.* **2000**, *8(3)*, 563-570.

13) A. Heine[#], G. DeSantis[#], J. G. Lutz, M. Mitchell, C.-H Wong, **Observation of Covalent Intermediates in an Enzyme Mechanism at Atomic Resolution** *(#* these authors contributed equally). *Science,* **2001**, *294*(5541), 369-374.

14) G. DeSantis, J. B. Jones **Combining Site-Specific Chemical Modification with Site-Directed Mutagenesis: A Versatile Strategy to Move Beyond the Structural Limitations of the 20 Natural Amino Acid Side Chains in Protein Engineering**. [Invited Review] *Methods in Molecular Biology (In Vitro Mutagenesis Protocols) 2nd ed.*, **2002**, *182*, 55-65.

15) G. DeSantis, Z. Zhu, W. A. Greenberg, K. Wong, J. Chaplin, S. R. Hanson, B. Farwell, D. P. Weiner, L. A. Nicholson, C. L. Rand, D. E. Robertson, M. J. Burk. **An Enzyme Library Approach to Biocatalysis: Development of Nitrilases for Enantioselective Production of Carboxylic Acid Derivatives**. *J. Am. Chem. Soc.* **2002**,*124*, 9024-9025.

16) J. Liu, G. DeSantis, C.-H. Wong **Structure –based rationalization of aldolase-catalyzed asymmetric synthesis**. *Can. J. Chem.* **2002**, *80*, 643-645.

17) G. DeSantis, **Enzymes in Organic Synthesis: Tools to Make Chiral Drugs.** [Invited Review] *Modern Drug Discovery, August 2002, 43-47.*

18) M.G. Silvestri, G.DeSantis, Michael Mitchel, C.-H. Wong. **Asymmetric Aldol Reactions Using Aldolases**. [Invited Review] *Topics in Stereochemistry*, **2003**, *23*, *267-342.*

19) G. DeSantis, J. Liu, D. P. Clark, A. Heine, I. A. Wilson, C.-H. Wong **Structure-Based Mutagenesis Approaches Toward Expanding the Substrate Specificity of D-2-Deoxyribose-5-phosphate Aldolase**. *Bioorg. Med. Chem* **2003**, *11*, 43-52.

20) G. DeSantis, K. Wong, B. Farwell, K. Chatman, Z. Zhu, G. Tomlinson, X.Tan, L. Bibbs, P. Chen, K. Kretz and Mark J. Burk **Creation of a Productive, Highly Enantioselective Nitrilase through Gene Site Saturation Mutagenesis (GSSM™),** *J. Am. Chem. Soc.* **2003**,*125,* 11476-11477.

21) D. E. Robertson, J. A. Chaplin, G. DeSantis, M. Podar, M. Madden, E. Chi, T. Richardson, A. Milan, M. Miller, D. P. Weiner, K. Wong, J. McQuaid, B. Farwell, L. A. Preston, X. Tan, M. A. Snead, M. Keller, E. Mathur, P. L. Kretz, M. J. Burk, J. M. Short, **Exploring Natural Protein Sequence Space For Enantioselective Catalysis.** *Applied and Environmental Microbiology,* **2004, 70(4),** 2429-2436

22) M. Burk, N. Barton, G. DeSantis, W. Greenberg D. Weiner, L. Zhao **Combining Enzyme Discovery and Evolution to Develop Biocatalysts for Chiral Fine Chemical Production** *Handbook of Chiral Chemicals, in press.*

Scientific Presentations:

1) P. Berglund, M.R. Stabile, G. DeSantis, W. G. Lai, M. Gold, J. B. Jones, C. Mitchinson, R.R. Bott, and T. P. Graycar; **Probing the Specificity of Subtilisin *B. lentus* by Combining Protein Engineering and Site-Specific Chemical Modification.** (poster) Gordon Conference: Biocatalysis, Meriden, NH. July 7, 1996.

2) G. DeSantis and J. B. Jones, **Tailoring the Specificity of subtilisin *Bacillus lentus* by a Combination of Site Directed Mutagenesis and Chemical Modification**. Genencor International, Inc. Palo Alto, CA. Oct. 17, 1996.

3) G. DeSantis and J. B. Jones, **Tailoring Subtilisin Properties by Combined Site-Directed Mutagenesis and Chemical Modification: An Update** Genencor International, Inc. Palo Alto, CA. June 5, 1997.

4) G. DeSantis and J. B. Jones **Tailoring the Specificity of the S_1 and S_2 Subsites of Subtilisin *B. lentus* Employing the Combination of Site-Directed Mutagenesis and Chemical Modification**. Hart House Farm Graduate Student Chemistry Retreat, Toronto, ON. May 22, 1998.

5) G. DeSantis, M.R. Stabile, P. Berglund, X. Shang, J. B. Jones **Altering the Catalytic Properties of Subtilisin Using the Combination of Site-Directed Mutagenesis and Chemical Modification**.(poster) Gordon Conference: Biocatalysis, Meriden, NH. July 6, 1998.

6) G. DeSantis, **Tailoring Enzymes Properties Via Chemistry and Mutagenesis**. Diversa Corporation, San Diego, CA. [Invited] Sept. 30, 1999.

7) G. DeSantis, A. Heine, M. Mitchell, J. Lutz, I. A. Wilson, C.-H., Wong. **Catalytic Mechanism of D-2-Deoxyribose-5-Phosphate Aldolase and Directed Evolution Approaches to Alter its Specificity.** American Chemical Socitey National Meeting, 221st, San Diego, CA April 1–5, 2001

8) G. DeSantis **Tailoring Enzymes Through Chemistry and Biology**, Canadian Society for Chemistry Meeting, 86th, [Award Lecture], Montreal, PQ May 28 2001.

9) G. DeSantis, Z. Zhu, W. A. Greenberg, K. Wong, B. Farwell, S. R. Hanson, A. Milan, J. Chaplin, D. P. Weiner, P. Chen. B. Morgan, D. E. Robertson, M. J. Burk. **Diversa's Discovery Point nitrilase Platform Enantioselective Production of Carboxylic Acid Derivatives**. (poster) Industrial Applications of Biocatalysis:, San Diego, CA. March 21, 2002.

10) G. DeSantis, Z. Zhu, W. A. Greenberg, K. Wong, B. Farwell, S. R. Hanson, A. Milan, J. Chaplin, D. P. Weiner, P. Chen. B. Morgan, L. A. Nicholson, C. L. Rand, D. E. Robertson, M. J. Burk. **An Enzyme Library Approach to Biocatalysis: Development of Nitrilases for Enantioselective Production of Carboxylic Acid Derivatives**. (poster) Gordon Conference: Biocatalysis, Meriden, NH. July 8, 2002.

11) G. DeSantis. **An Enzyme Library Approach to Biocatalysis Development.** Enzymes and Biocatalysis for Drug Discovery and Development, San Diego CA, Jan 30-31, 2003.

12) G. DeSantis, **Nitrilases for Chiral Synthesis: Discovery and Evolution**. Society for Industrial Microbiology, Minneapolis MN, Aug. 10 - 14, 2003.

13) G. DeSantis, **Nitrilase Discovery and Evolution: Accessing Chirality for Pharmaceutical Intermediate Development,** Biocatalysis for Pharmaceutical Production, Boston MA, Oct. 13 - 15, 2003.

14) G. DeSantis, David P. Weiner **Nitrilase Discovery and Evolution: Strategies and Insights for Accessing Chirality,** Gordon Conference: Biocatalysis, Meriden, NH. July 13, 2004.

Patents:

R. R. Bott, T.P. Graycar, C. Michinson, G. DeSantis, J. B. Jones, Bacillus lentus Subtilisin Mutants with Chemically Modified Cysteine Residues, PCT Int. Appl. **1998** 26 pp. PIXXD2, WO9823732 A2 19980604, International.

Burk, M. J.; Desantis, G.. Peptide synthesis method. PCT Int. Appl. (2002), 41 pp. CODEN: PIXXD2 WO 0294855

Madden, M.; Desantis, G.; Chaplin, J. A.; Weiner, D. Paul; Milan, A.; Chi, E.; Short, J. M.; Burk, M. Bacterial nitrilase and gene sequences exhibiting stereoselectivity useful for synthesis of chiral reaction products. PCT Int. Appl. (2003), 560 pp. CODEN: PIXXD2 WO 0300840

Several additional patent applications underway.

Societies and Committees:

2002 - 2004	Referee, Biotechnology & Applied Biochemistry
2002 - 2004	Member, Southwestern College Chemical Technology Program Advisory Alliance
1995 - 2004	American Chemical Society
1994 - 2003	Canadian Society for Chemistry
1998 - 2000	Toronto Biotechnology Initiative
1997 - 1998	Graduate Student/Post Doctoral Fellow Advisory Committee (U of T)